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Memoirs of the Department of Agriculture in India

STORAGE

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BY

E. J. BUTLER, M.B., F.L.S

Imperial Mycologist

AND

ABDUL HAFIZ KHAN

Assistant to the Imperial Mycologist

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PUBLISHED FOR

THE IMPERIAL DEPARTMENT OF AGRICULTURE IN INDIA

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PLATE I.

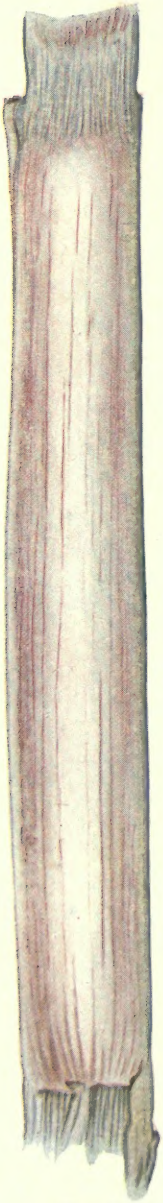

Fig. 1.

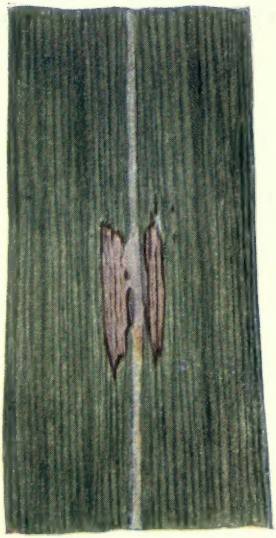

Fig. 3.

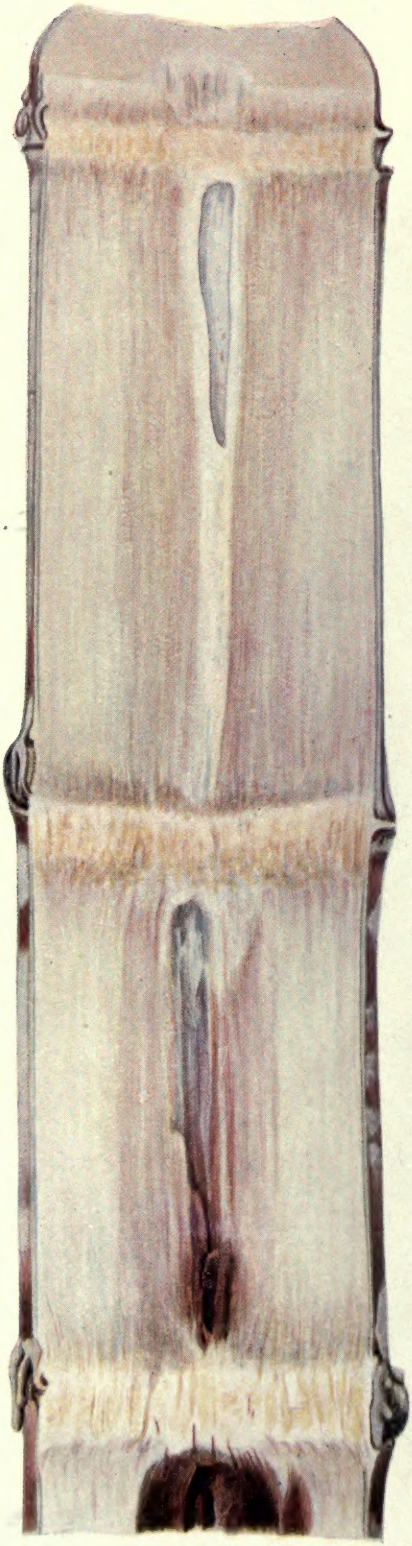

Fig. 2.

DESCRIPTION OF PLATE I.

Fig. 1. Appearance of split cane infected with *Cephalosporium Sacchari*. Natural size.

„ 2. Appearance of split cane infected with *Hendersonina Sacchari*. × 1½.

„ 3. Leaf spot caused by *Helminthosporium Sacchari*. Natural size.

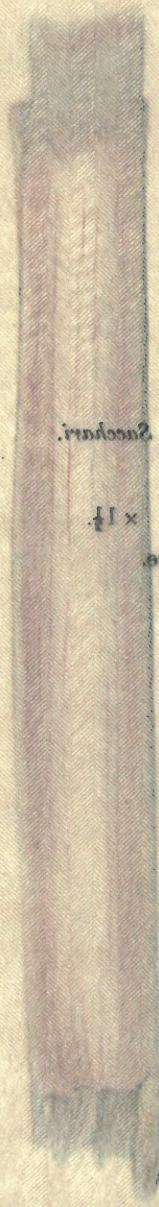

Fig. 1.

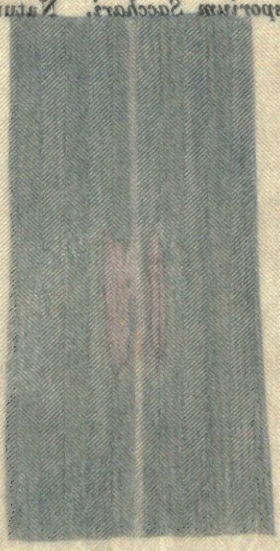

Fig. 3.

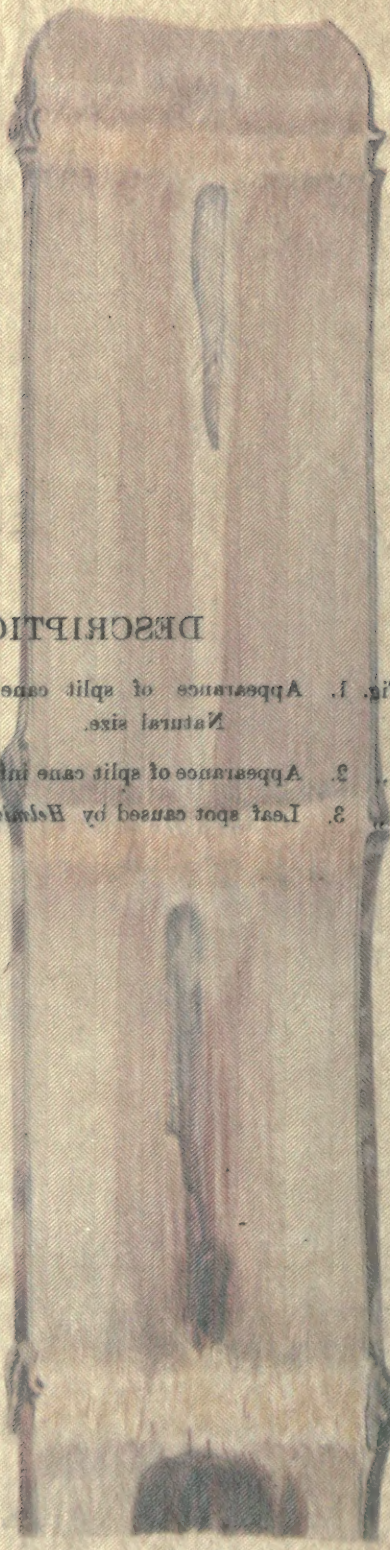

Fig. 2.

SOME NEW SUGARCANE DISEASES

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I.—Wilt.

(*Cephalosporium Sacchari* Butl. n. sp.)

In the course of investigations of the red rot of sugarcane, caused by *Colletotrichum falcatum* Went, a second disease, to which the name red rot might equally well be applied, since it produces distinct reddening of the cane pith, has frequently been encountered. With a little practice the two diseases can be distinguished and they are caused by entirely distinct organisms.

As in red rot, the earlier symptoms are elusive. Except in such cases of severe infection of the cuttings selected for planting as cause the death of the young shoots, in the manner more fully referred to below, little is noticed until the cane is about half grown. At this period affected canes lag in growth and stunted, single stools, or patches of varying size, may soon be observed scattered through the fields in which the disease is prevalent. From this on until the time of harvest, withering of individual canes, or even of whole stools, occurs. The leaves dry up, as if insufficiently supplied with water, followed by the stems, which become light and hollow. If the cane be split longitudinally when the leaves are first observed to wither, a charac-

teristic discoloration of the pith, as shown in Pl. I, Fig. 1, may be observed. A comparison of this figure with a coloured drawing of true red rot* will show the difference in appearance of the two diseases. Instead of bright red patches and streaks, broken by transversely expanded white areas, there is a diffuse purple or dirty-red colouration, in which brighter red, vertical lines mark the position of the bundles. The tendency of the colour to become muddy at an early period is its most strongly marked character and serves to distinguish the disease from any other known to us. In old cases the red almost disappears, being replaced by an earthy brown. The pith dries up more rapidly than when attacked by *Colletotrichum* and becomes hollow. Within the hollow portion, a fluffy grey growth of mould is often found.

Microscopic examination shows that the stem is infested throughout the reddened portion, which may be confined to a few joints or extend to the whole length of the cane, by a fungus, whose hyphæ ramify through the cells in all directions, penetrating the fibro-vascular bundles as freely as the large-celled parenchyma of the pith (Pl. II, Figs. 2 and 3). Most of the hyphæ are fine, not exceeding 3 μ in diameter in recently infected parts, and either uniform or tapering gradually. They pass from cell to cell through the pits in the walls, when pits are present (Fig. 2), but can also penetrate unbroken walls and seem to have no preference for one tissue more than another. Even when old, they remain hyaline. Occasionally they have been found bearing conidia in the vessels (Fig. 5) and possibly do so also in the parenchyma of the pith, as the condition shown in Fig. 4 appears to represent an early stage of spore-formation. The conidia are small, hyaline, unseptate, oval, and usually appear to be borne singly on short lateral branches of the mycelium. They resemble closely the microconidia frequently found in the vessels of plants affected with the *Fusarium* wilts.

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* See Memoirs, Dept. of Agric. in India, I, No. 3, 1906, Pl. I, or Agric. Journ. of India, II, 1907, Pl. XI.

In the hollow which invariably forms in the pith of affected internodes at a late stage in the disease, there is usually a copious greyish-white, fluffy growth of hyphæ, similar to those found in the tissues, and bearing great numbers of conidia, resembling those observed in the vessels, though often of somewhat larger size. The characters of the fungus can be studied from this growth or, more satisfactorily, from pure cultures, and they show it to be a member of the form-genus *Cephalosporium*, several species of which are known to be lower stages in the development of *Hypocreaceæ*.

The hyphæ are hyaline, slender (about 3 to 5 μ in diameter), richly branched, sparingly septate when young, not varying abruptly in diameter, though swollen segments are found in old cultures. Sometimes the individual filaments unite together to form coremial strands, usually only two or three hyphæ taking part in the formation. An early stage of such a strand is shown in Fig. 6*a*. Chlamydospores have not been observed either in culture or in diseased canes.

The conidia are borne on short, simple or branched, lateral hyphæ and also terminally on the ultimate branches of the mycelium. They measure 4 to 12 μ (usually 5 to 8 μ) by 2 to 3 μ, when formed, but increase in size prior to germination. Their shape varies from shortly oval to ovoid or long elliptical. Occasionally they are curved or with one side flattened. They are almost invariably unicellular when formed, but some become septate prior to germination, the septa being 1 to 3 in number (Fig. 11). Spores and mycelium are hyaline.

The conidiophores are usually of definite shape, measuring from 6 to 30 μ by 3 to 4 μ at the thickest part, swollen slightly in the middle or lower third, narrowing below where they arise from the mycelium and tapering above, but with an obtuse apex on which the spores are borne (Fig. 7). They may be scattered along the hypha or arise in bunches near together (Fig. 6). Branching is not uncommon, being irregular or once whorled or forked. Each branch usually bears conidia at the apex, but if kept very moist the branches may grow out into long hyphæ.

The first conidium is borne at the tip of the conidiophore and, when full grown, is pushed to one side by the formation of a second spore at the same point, and so on until a number have been produced. Sometimes, instead of forming a second spore, the apex continues growth as a hypha, which may again bear conidiophores (Fig. 13*a*). Unlike most members of the genus, the successively formed conidia are not held together in a mucilaginous drop, but adhere merely by surface attraction and are very easily dislodged. If kept free from currents of air in a moist chamber, heads of 5 or more can readily be found. They adhere more firmly at the base than elsewhere and if carefully mounted, after treatment with acetic acid, such appearances as those figured in Fig. 7*a* can be observed. At first sight this suggests that the spores arise near together and at the same time, as in the genus *Haplotrichum*, but more careful examination shows that this is not the case, only one spore being formed at a time and the whole apex of the conidiophore taking part in its formation. The apex is pushed out by growth from within the hypha to form the spore, and if examined just after the latter has fallen and before a second appears, the condition shown in Fig. 9 is found. In moist-chamber cultures spores formed on the aerial mycelium appear as shown in Fig. 8, lying in a single layer with their long axes parallel. Spore-production is extraordinarily copious, almost every hypha being abundantly provided with conidiophores throughout the greater part of its length.

Germination occurs within 24 hours in many cases, the large septate and small unseptate conidia behaving in much the same manner (Fig. 11). The germ-tube is usually single and terminal, but the septate spores may give out a tube from each end. Union of two or more germ-tubes, belonging to different spores, is common (Fig. 12), up to five spores having been found united in this manner. One or two of the germ-tubes grow out into strong hyphæ from the united mass, the arrangement being probably merely a nutritional adaptation.

In culture on nutrient agar the mycelium remains white and filamentous and no formation of stromata or of other types of

spores has been observed. The species does not agree with any previously described and has been named *Cephalosporium Sacchari*, the diagnosis being as follows :—

Cephalosporium Sacchari Butl. n. sp. Effusum, candidum ; hyphis repentibus, parce septatis, 3-5 μ diam. ; conidiophoris continuis, simplicibus furcatis vel subverticillato ramosis, sursum obtusis, medio vel versus basim incrassatis, 6-30 μ long. 3-4 μ crass.; conidiis ex apice ramulorum pluribus continuis exsilientibus et in capitula collectis sed facillime secedentibus, hyalinis, ovoideis vel oblongo-ellipsoideis, continuis, 4-12 $\times$ 2-3 μ.

In culmis *Sacchari officinarum* in India or

Cultures of the fungus can be readily obtained in the same way as with *Colletotrichum falcatum*, by cutting out aseptically a portion of the pith, in the early stage of the disease, and incubating in a sterile Petri dish or potato tube. The growth is frequently pure from the commencement, but if not, can be readily purified by sub-culturing from the aerial mycelium. The following inoculations were carried out with pure cultures from nutrient agar, in which spore-production was copious.

On December 13th, 1908, 20 strong sound Red Mauritius canes were inoculated in a stem wound with a suspension of spores and mycelium in distilled water, from a culture two weeks old. The inoculations were made in the lower half of the cane by removing a cylinder of pith with a small sterile cork borer, inserting a drop of the suspension, and replacing the cylinder after cutting off a little of the end, so as to leave a small cavity. The wound was then bound with sterile gutta-percha sheeting.

The canes were examined after 3 months. In one case only had the whole cane dried up, the infection extending above the wound to the top of the cane, and below for 3 internodes. In the others infection had progressed through from 4 to 6 internodes. The inoculated internode had become hollow in every case, those above and below being also hollow in some cases. The symptoms were typical and the fungus was found throughout the discoloured parts.

On the same day 15 canes were inoculated at leaf scars, the scar being covered after inoculation with a wad of damp sterile cotton-wool, bound with gutta-percha sheeting. The same material was used as in the last experiment.

These inoculations also succeeded, distinct reddened bundles being found after 3 months, extending downward from the scar through the next internode. The progress of the infection was less than in the last case and could not be traced beyond the one internode. The hyphæ were found chiefly in the reddened bundles and there was not much lateral spread. It was noticed that shoots arising from the inoculated nodes were not infected.

The fungus cannot penetrate the unbroken rind of the internodes. Sixteen inoculations were made to test this, a drop of the suspension being placed on the rind, and covered with damp sterile cotton-wool for 48 hours. In no case was there the slightest sign of infection.

As in *Colletotrichum falcatum*, however, the adventitious root eyes at the nodes can be inoculated successfully. Ten inoculations were made on the uninjured eyes, in exactly the same way as in the last experiment, except that the cotton-wool was removed after 36 hours. The first sign of success was noticed after 4 days ; one of the eyes being slightly discoloured. On sectioning, the hyphæ were found to have penetrated through the epidermis and to have reached almost to the centre of the bud. Three days later all the inoculations had clearly succeeded, the eyes being reddened and full of hyphæ. After 10 or 11 days the eyes were quite killed and the hyphæ were passing into the main stem.

To see if the cane can be infected through the setts at the time of planting, half a trench of Striped Mauritius was planted on March 7th, 1907, after dipping the cut setts in a suspension of spores and mycelium in distilled water. The remaining half trench served as control. Germination was approximately equal in the two halves, but 22 shoots withered in the inoculated half up to June 17th, whereas there were only a few deaths in the other

half, apparently due to white-ants. The *Cephalosporium* was recovered from 3 of the withered shoots examined.

As this fungus has been several times found associated with *Colletotrichum falcatum*, experiments were made similar to the last, but with mixed suspensions of the two fungi, in 1907. The results were striking. Germination was good in both halves of the trench, but 45 shoots withered in the inoculated half in the first three months, while there were very few failures in the rest of the trench. *Colletotrichum* was recovered from 2 of the withered shoots and *Cephalosporium* from 8, out of 14 examined. The experiment was repeated in 1908 with much the same result.

These experiments show that the fungus can enter the cane through wounds, through the uninjured root eyes at the nodes, and through the planted setts. In true red rot the writers have shown* that the latter is the most frequent method of infection in Northern India. In the present case the evidence is not so complete, but it has been found that wound infection is far more common than with *Colletotrichum*. The wounds examined have been all borer holes, which are much the most frequent wounds in standing cane. Altogether 27 have been examined by incubating an aseptically removed slab from just beyond the margin of the hole and, of these, 15 have given the fungus. Out of 9 examined in the 1908 crop when nearly fully grown, 8 were infected. This was the year when the most widespread attack of *Cephalosporium Sacchari* was observed at Pusa.

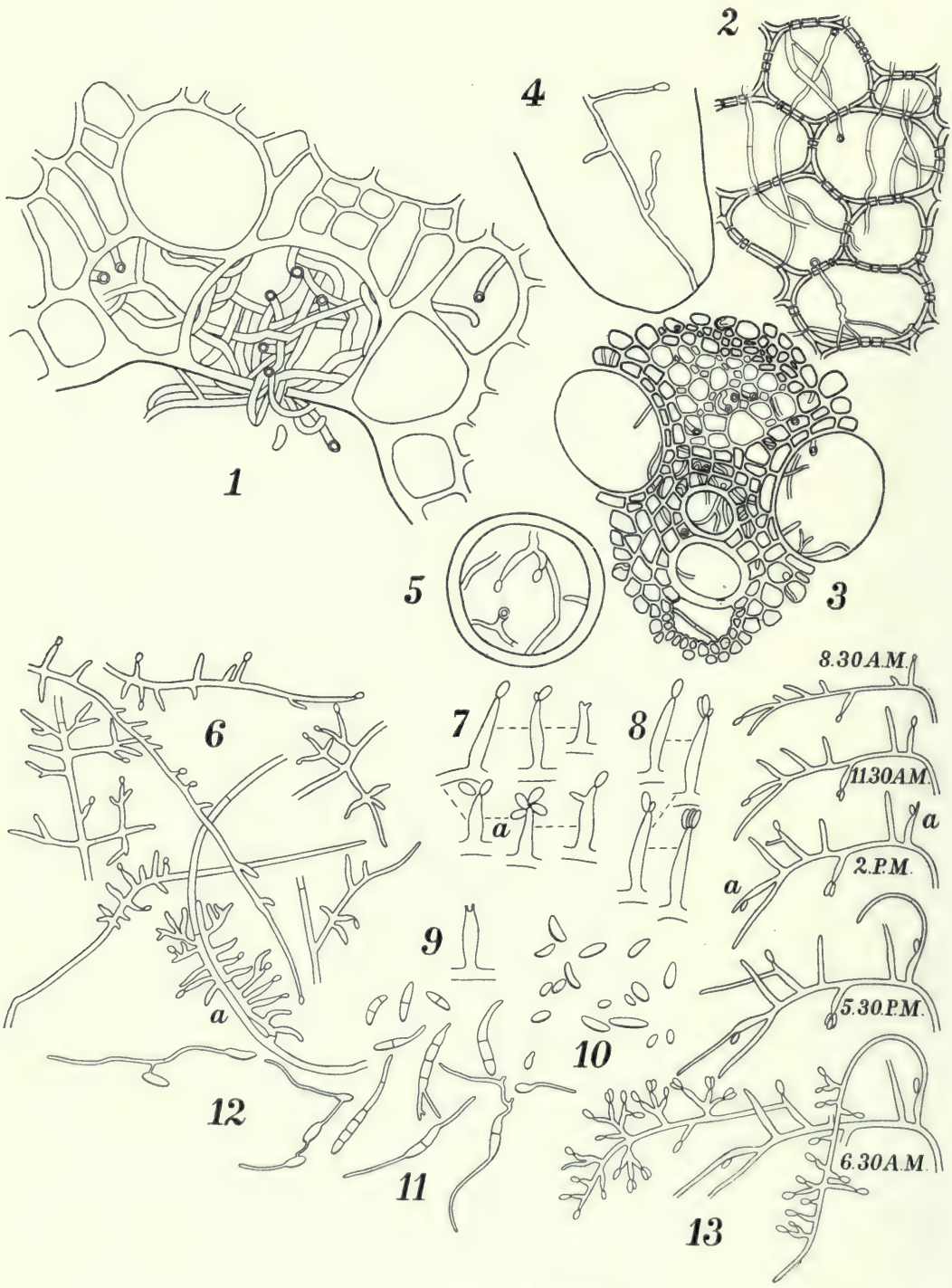
The disease is found over a large part of India. It has been observed at Surat, Poona, Samalkota and throughout North Eastern India. As a rule it seems to accompany red rot, quite a considerable percentage of the cases examined being due to mixed infection. In the 1908 attack at Pusa, however, there was practically no true red rot and a good deal of damage was done by an unmixed attack of *Cephalosporium*. Similarly, in 1912, several cases of pure infection were encountered, and pure cultures were obtained direct from the mycelial growth in the

* Butler, E. J., & A. Hafiz Khan, Red Rot of Sugarcane. Mem. Dept. of Agric. in India, Bot. Ser., VI, No. 5, 1913.

hollows of the pith. As a rule, so far as has been observed, the infection is not virulent; spread within the cane is gradual and communication from one plant to another slow. A large number of borer holes become infected late in the season, but the parasite usually seems to remain confined to a few internodes. As bored setts are usually discarded at planting time, infection by this channel is not often transmitted to the following crop. The fungus has not been found on the leaves or on the surface of the stem, in the field. In the laboratory, however, a case of spontaneous infection of the leaf with a fungus exactly resembling that under consideration occurred, and this led to further examination. Inoculations were made with cultures obtained from the growth on the leaf and also with cultures from diseased cane stems. Both gave similar results. Six inoculations were made with each of the two cultures. In two cases the inoculated leaf withered too rapidly to allow of definite observations. The remaining ten were quite successful, Pl. II, Fig. 1, showing the condition after three days. Penetration had occurred chiefly through the large thin-walled motor cells. We have not been successful in finding cases of leaf infection in the field, but as leaf spots are extremely common on sugarcane such a search is not easy. The spot caused by *Cephalosporium Sacchari* in artificial inoculations resembles that caused by *Helminthosporium Sacchari*, described in a later section of this paper, and all the field cases examined belonged to this latter fungus.

Rotten canes must set free large quantities of spores and the source of the infection of cane wounds may come from these, from the leaves and, if the species passes part of its life as a soil saprophyte, as is not improbable, possibly also from the soil. The genus is so common in cultivated soils, and this species has so little to distinguish it from some of the habitual soil dwellers, that its recognition would be difficult. It lives very readily as a saprophyte, however, growing luxuriantly on most of the common culture media and remaining alive in culture for about 3 months, so that it is probably present in the soil of cane fields. The inoculation experiments indicate that infection of the setts at the

PLATE II.



CEPHALOSPORIUM SACCHARI.

time of planting, causes a good many of the young shoots to wither within the first 3 months. As this early withering has not been very frequently observed in natural attacks, we conclude that infection of the growing cane, through wounds and through the root buds at the nodes, is the more common origin of the disease.

The control of the disease should evidently be on much the same lines as in ture red rot*. As, however, wound infection is far more common, the importance of removing diseased clumps before they have time to rot and set free spores is much greater. As a rule the disease is not a severe one and though our experience with it is limited as yet, it is probable that it is incapable of doing permanent damage so long as the measures advocated against red rot, which we consider to be essential to the successful growing of thick cane in Northern India, are carried out.

Description of Plate II.

(*Cephalosporium Sacchari* Butl.)

Fig. 1. Leaf of sugarcane inoculated with *Cephalosporium Sacchari*, showing fungus in the motor cells and emerging to the surface. × 640.

„ 2. Hyphæ in parenchyma of stem and passing from cell to cell through the pits in the walls. × 190.

„ 3. Hyphæ in fibro-vascular bundles. × 190.

„ 4. Hypha in a cell of the parenchyma with indications of spore formation. × 320.

„ 5. Spore formation on hyphæ in a vessel of the stem. × 320.

„ 6. Mycelium bearing conidia, from a pure culture on nutrient agar. At *a* the commencement of the formation of a coremial strand is seen. × 320.

„ 7. Conidiophores from a pure culture, after treatment with acetic acid. At *a* the adherence of the conidia at their bases is indicated. × 640.

„ 8. Successive stages in conidial formation, drawn from a continuous observation. × 640.

„ 9. Conidiophore immediately after a spore has fallen and before a new one has commenced to appear, showing the hollowed apex. × 640.

„ 10. Conidia from nutrient agar culture, 6 days old. × 640.

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* Butler, E. J., & A. Hafiz Khan, *loc. cit.*

Fig. 11. Germination of the conidia, showing especially the septation which sometimes precedes germination. × 320.

,, 12. Union of the germ-tubes of the conidia. × 320.

,, 13. A spore-bearing hypha continuously observed, showing the extent of growth and spore formation in 22 hours. At *a* conidiophores have continued apical growth after forming a single conidium. The septa have been omitted.

II.—Collar Rot.

(*Hendersonina Sacchari*, Butl. n. g., n. sp.)

In the last section a disease was described which has frequently been mistaken for red rot. A second disease is sometimes confused with red rot in India, and the following description may lead to its recognition and thus secure further information regarding its distribution and the amount of injury it causes the crop, points on which our observations are incomplete.

Towards the end of 1908, a number of canes withered at the Samalkota Sugarcane Station, following on a cyclone at the end of September, which did much harm to the crop. A recrudescence of red rot was feared, as many of the canes were reddened at the base. We examined the crop in January, 1909, and found extremely little true red rot. Some of the damage was apparently not caused by disease but by injury to the root system as a result of the storm. A good many cases were seen, however, in which the symptoms suggested the action of a definite parasite at the base of the plant, and a fungus was isolated from the tissues in these cases which proved capable of reproducing the disease.

In the following season the disease reappeared and was more fully studied, but for the last three years there have been no further records of its occurrence on this farm. Recently it has been found at Jorhat Farm in Assam, and it is possible that it also exists in the Central Provinces, where we have observed a somewhat similar condition, but without being able to make a detailed examination. It has not been recorded elsewhere, and the parasite differs so widely from any previously known that it has been necessary to make it the type of a new genus. The varieties of cane so far observed to have been attacked are Red Mauritius, Striped Mauritius and B. 208.

The symptoms outwardly resemble those of red rot, so far as the withering of the top is concerned. The top leaves wither back from the tip along the edges, the midrib remaining green later than the rest of the leaf. The larger leaves below the crown appear to suffer first, those at the apex remaining unaffected for some time. When the leaves have fully withered, the cane is found to be much lighter than normal. On splitting, the upper part is usually pithy and dry in the centre, or even with a central cavity along each internode, around which the pith is dry, white and flaky. Lower down the pith may be still juicy but has a curious translucent watery appearance ; still lower the centre portion may be brown, while red streaks or patches may often be seen, especially at the nodes (Pl. I, Fig. 2). At the base, where the feeding roots arise, the red colour predominates and is especially visible at the nodes. In old cases the lower internodes also dry up and may develop a central cavity, surrounded by red or brown pith. The roots arising from the basal nodes are usually blackened and rotten, and the appearance suggests that the disease enters the base of the stem from the roots.

In the Jorhat cases the buds at the lower nodes had sprouted and subsequently withered. The reddening was specially well developed at the nodes from which these shoots arose. It is not yet certain how far this sprouting of lateral buds is a symptom of the disease, as it appears possible that the Jorhat attack is complicated by the occurrence of a non-parasitic, *sereh*-like degeneration of the cane, which requires further investigation.

Microscopic examination shows that the roots and base of the stem are invaded by a fungus. The mycelium is confined to the definitely reddened parts, being absent from the translucent upper portion of the cane. In the Jorhat cases the fungus was found collected chiefly at the nodes, the intervening internodes being almost free from hyphæ until a late stage. The discoloured roots always contain a considerable quantity of mycelium. In the early stages the hyphæ run between the cells in the intercellular spaces. Here they are usually extremely fine, though thick ones sometimes occur. Later on, branches from the inter-

cellular mycelium penetrate the walls to enter the cells. All the tissues are invaded, bundles as well as parenchyma (Pl. IV, Fig. 1). In the cells, and especially in the vessels, very thick hyphæ may occur, sometimes almost alone, sometimes intermingled with the fine filaments. Careful observation shows that both kinds belong to the same mycelium and can be found in direct continuation with one another. Sometimes a haustorium-like, branched mass, arising from thick hyphæ in an intercellular space, almost fills a cell. At first septation is rather scanty, but in the older hyphæ the septa lie very close together, the segments thus formed being often broader than they are long. No trace of spore-formation was found either in the tissues or on the surface of the diseased canes before their death.

Cultures of the fungus were obtained in the usual way, from the surface of the cane pith cut with a red-hot knife. The fungus grew well on ordinary nutrient agar, forming a dense superficial growth. The following description is from pure cultures on this medium.

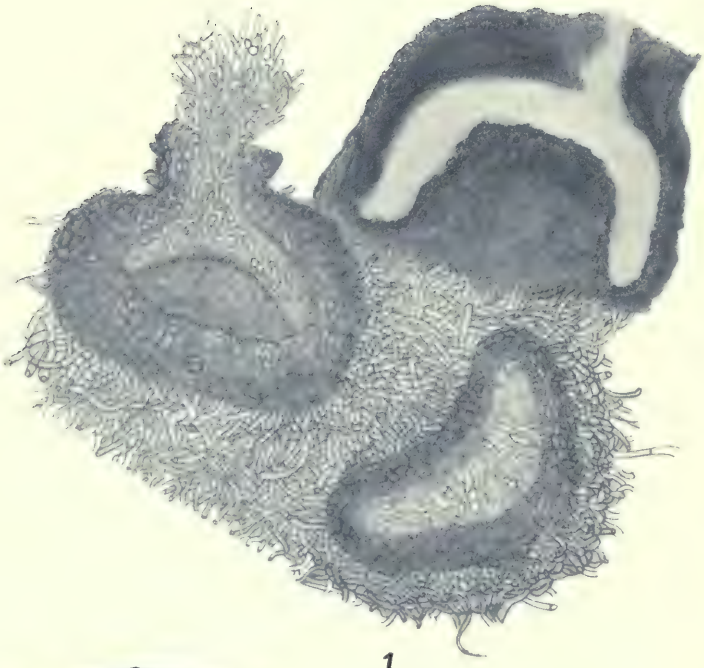
The mycelium is white or faintly tinged yellow. The hyphæ (Pl. IV, Figs. 2 & 3) are very variable in size. The main branches are very thick, sometimes up to 15 μ in diameter, at first hyaline and sparingly septate, then pale yellowish and closely septate. Branching is copious and often rectangular. Very noticeable is the tendency of the older branches to give off extremely fine, hyaline, thin-walled hyphæ, the difference in diameter being so great as to suggest a distinct mycelium, until carefully examined. The thin hyphæ are often irregularly swollen or even nodular and measure sometimes as little as 1 μ in diameter. Anastomosis of neighbouring branches is not uncommon (Pl. IV, Fig. 3*a*). Intermediate stages, consisting of hyphæ 6 to 8 μ in diameter, freely septate when old and often irregularly swollen, are common. The thicker branches break up readily into chlamydospores (Pl. IV, Figs. 4 & 5), which may be terminal or intercalar, and are usually arranged in short chains. They consist of thick-walled cells filled with reserve material, round, oblong or long elliptical in shape, variable in size, the

largest up to 33 μ in diameter, at first hyaline but later slightly coloured. They frequently separate from the mycelium when mature, and may be found lying singly or in short chains intermingled with the hyphæ. They germinate in water by an outgrowth of the endospore. The nodular swellings on the smaller hyphæ seem to be of the same category, but do not usually become detached ; they often give off fine branches after reaching maturity. In many cultures no other spore form was found. In several, however, a pycnidial stage developed, and this stage was subsequently found on the surface of old canes which had been killed by the disease.

When pycnidia were formed, they began to appear in about 3 weeks after sowing the tubes, as fine blackish dots immersed in the white mycelium. These grew to form prominent black nodules, which, under the microscope, were found to consist of stromatic tissue with one or more sporiferous loculi entirely immersed in the stroma.

The stromata are leathery, roundish-conical, about 1 to 2 mm. in diameter, and consist of an outer portion of brown, fibrous tissue, composed of closely woven hyphæ, and an inner, bounding the loculi, of dark brown pseudo-parenchyma, many layers deep. Sometimes bands of the fibrous tissue separate the loculi, each cavity then having its separate investment of pseudo-parenchyma (Pl. III, Fig. 1), in others the whole centre portion of the stroma is cellular and the cavities have no distinct walls, except the lighter coloured layers from which the spore stalks arise. The cells of the pseudo-parenchyma are distinct, angular, isodiametric in the deeper layers, but becoming more elongated as the hymenium is approached, with unthickened but deeply pigmented walls and oily contents, and measuring up to 10 μ in diameter. The colour gradually becomes lighter towards the spore-bearing surface.

The pycnidial loculi are deeply sunken in the stroma, very irregular in shape, and often communicate with one another by narrow passages. Every stage may be found between stromata with a single loculus, through such cases as shown in Pl. III,



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Hendersonina Sacchari.

Photo. Engraved & printed at the Offices of the Survey of India, Calcutta, 1913

Fig. 1, which we take to represent the union of several unilocular stromata, to truly multilocular pseudo-parenchymatous masses. The cavity is usually narrow, due to cushion-like projections of the wall into the lumen. In small unilocular stromata, the cushion arises usually at the base, the cavity being convex outwards. In larger stromata cushions may also project from the sides, leading to very irregular formations. In transverse section through the middle of the stroma (Pl. III, Fig. 3), the cavities are quite irregular in position, but near the apex the arrangement may be roughly circular, due probably to several loculi converging to a common opening. Laterally situated loculi may, however, open by separate orifices. In longitudinal section (Pl. III, Fig. 2), there is no uniformity of disposition or size of the cavities. The mouth is formed late and is usually not prominent.

The spores are of two kinds, both borne on exactly similar sporophores. The latter arise from the innermost layer of the pseudo-parenchyma, which is not sharply marked off as a hymenium but consists simply of small, rather elongated cells, light yellow in colour (Pl. IV, Fig. 6). From these one or more hyaline sporophores arise and project into the cavity. Usually the sporophore is branched, the branches arising chiefly near the base and each terminating in a single spore (Pl. IV, Figs. 7 & 8). In some stromata unbranched sporophores predominate (Pl. IV, Fig. 9). Septa occur sparingly and the ultimate branches are always slender and unseptate. Each sporophore appears always to bear only one kind of spore, but as they are closely crowded at the base, and both spore forms are found side by side in the same cavity, it is difficult to be certain on this point. Both kinds of spores appear to arise simultaneously.

The most highly differentiated spore form (Pl. V, Fig. 1), consists of brown, elongated cells, rounded at the ends, unicellular or with one or two transverse septa, usually straight but sometimes curved, the curvature in bicellular spores being occasionally sigmoid. They measure 15-24 $\times$ 3·75 to 5 μ. In some cultures unicellular spores predominated, in others bicellular. Three-celled spores are the least numerous. Germination occurs

in less than 24 hours by an outgrowth from one or both ends, the germ-tube remaining unseptate until it has reached a considerable length (Pl. V, Figs. 4 & 5).

The other spore form (Pl. V, Fig. 2), consists of hyaline filamentous cells, usually without septa but with many oil drops, straight or irregularly curved, variable in length and breadth, the longest being often very narrow, sometimes broader at the base and tapering to a narrow apex, sometimes quite uniform in diameter. They measure 20 to 60 by ·6 to 2 μ. Germination was not observed.

An intermediate form of spore (Pl. V, Fig. 3) is sometimes found, consisting of pale yellow, elongated, 1-septate cells, borne on sporophores of the type above described and measuring 18 to 30 by 2 to 3·75 μ.

After the pycnidial stage had been obtained in pure cultures, it was found in two cases on old canes attacked by the disease, one being an inoculated cane at Pusa, the other a diseased cane from Jorhat. The stromata develop in the internodes, under the epidermis, or one or two layers deeper in, and are smaller than those obtained in culture. The outer fibrous layer is absent or reduced, the mass of the stroma being parenchymatous. The base is broad and extends for some distance all round, as a narrow stromatic layer between the tissue cells. In the centre, the epidermis is raised up and ruptured by the roughly conical, deep portion of the fruit body, which is hollowed out into one or several cavities. Unilocular stromata are common, the cavity being irregular or sometimes imperfectly divided by incomplete walls. The mouth is usually single and develops late. Both spore forms occur in the loculi.

There is much greater uniformity in the spore characters in any one culture of the fungus, than would appear from the above description, which is based on the examination of a large series. Thus, in some cases, the first spore form was almost entirely unseptate and small and the second broad and distinct; in others bicellular spores of the first type were chiefly found, intermingled with which were a few hyaline, attenuated spores of the second

type ; in others again the narrowly filiform second type predominated, while there were numerous "intermediate" spores and only a few typical bicellular brown spores ; finally in some cases, especially in the earlier cultures, all the types above described, except the "intermediate," were found, though 3-celled spores were never common.

Hence we have had some difficulty in deciding whether the fungus should be considered to belong to the sections *Phœodidymæ* or *Phæophragmiæ* of the *Sphærioidaceæ*. But the characters as a whole agree better with those of the latter section, of which *Hendersonia* is the main type, than with the former, which consists mainly of *Diplodia* and its allies, and the 3-celled brown spores should, we think, be considered as the most highly differentiated and, therefore, most important for systematic purposes. The genus differs from the rest of the *Phæophragmiæ* chiefly in the characters of the stroma and the possession of two distinct types of spore in the same loculus. The latter peculiarity approaches it to the genus *Phomopsis* amongst the *Hyalosporæ*, and it is of great interest as supporting the view that the hyaline, filamentous bodies of the latter genus are true spores, and not merely disjuncted basidia as has been maintained by some observers. There cannot be the smallest doubt that these bodies in the present fungus are spores, for they are borne on true sporophores, in an exactly similar fashion to the spores of the first type. Another fungus in which two spore forms are found in the same pycnidium is *Fusicoccum viticolum* Red*. ; which Shear† has shown to be the imperfect form of *Cryptosporella viticola* Shear. Shear distinguishes the two spore forms as pycnospores and scolecospores, and believes the latter to be true spores, not basidia as held by Reddick. The pycnidia themselves are immersed in a stroma, which is evidently of much the same type as that of the sugarcane fungus.‡ It should be

* Reddick, D. Necrosis of the grape vine, Cornell Univ. Agr. Expt. Stat. Bull. 263, 1909.

† Shear, C. L. The ascogenous form of the fungus causing dead-arm of the grape. Phytopathology, I, 1911, p. 116.

‡ Gregory, C. J. A rot of grapes caused by *Cryptosporella viticola*. Phytopathology, III, 1913, p. 20, fig. 1.

noted that several species described as *Phomopsis* are multilocular stromatic*, though Diedicke, who has most fully studied the genus, places it amongst the simple forms, and does not seem to include any species with a multilocular stroma. Another genus which may be compared is *Endothiella* Sacc., where the bodies described as filiform basidia or pseudo-paraphyses are probably scolecospores.

The following is the diagnosis :—

Hendersonina Butl. nov. gen. *Sphæropsidacearum*—Stromata innato-erumpentia, atra, coriacea, parenchymatice contexta. Pycnidia (loculi) immersa, inæqualia, ostiolis sæpe confluentibus. Basidia ramosa. Sporulæ in basidiis acrogenæ, alteræ fuligineæ, ellipticæ vel elongatæ, rectæ vel curvulæ, continuæ vel 1-2 septatæ, alteræ hyalinæ, filiformes, rectæ vel flexuosæ, continuæ.

Hendersonina Sacchari Butl. n. sp. Stromatibus cortice innatis demum erumpentibus, subgloboso-conicis, 1-2 mm. diam., atris, intus 1-pluri-locularibus ; loculis irregularibus, subinde incompletis vel inter se communicantibus, ostiolis sæpe confluentibus; contextu brunneo, minute parenchymatico ; basidiis ramoso-fasciculatis, hyalinis ; sporulis dimorphis, aliis fuligineis, rectis vel curvulis, ellipsoideis vel elongatis, utrinque obtusis, continuis vel 1-2 septatis, 15-24 ⩒ 3·75-5 μ, aliis hyalinis, filiformis, rectis vel flexuosis, pluriguttulatis, 20-60 ⩒ ·6-2 μ.

In culmis *Sacchari officinarum* in India, or

As this fungus is possibly the lower stage of a Pyrenomycete, attempts were made to obtain an ascigerous stage in culture on organic media. The following were tried :—Potato, carrot, onion, plantain, *Colocasia* corm, fruit of *Carica Papaya*, fruit of *Psidium Guajava*, boiled rice, bread paste and sugarcane pith. The last medium was the only one, besides nutrient agar, in which pycnidial stromata were developed and none gave rise to

* *c.f.* Harter, L. L. & Ethel C. Field. *Diaporthe*, the ascogenous form of sweet potato dry rot. Phytopathology, II, 1912, p. 121. Roberts, J. W. A new fungus on the apple. *ib.*, p. 263.

an ascigerous stage. After a week the onion cultures were the best, followed closely by carrot, potato, *Carica*, *Colocasia*, *Psidium* and rice. On bread also the growth was good, while plantain and sugarcane were much inferior, especially the last. The mycelium was loose and had begun to lose its original white colour in many of the tubes, the upper part of the aerial mycelium (which exceeded 2 inches in height in some of the cultures) turning grey, except in the plantain, rice and sugarcane tubes, while the growth near the surface of the medium remained white in all. A submerged growth had developed in the water in the bottom of the (potato) tubes and this became light purple or pinkish in the carrot, onion and *Carica* tubes. The medium was discoloured dirty grey in the onion tubes, eye-grey to purple in those of rice and slaty-blue in those of bread. Five days later a blackish crust had developed on the surface of the water in the potato, carrot, onion, *Carica* and *Psidium* tubes, the submerged mycelium was light cinnamon colour in the onion and *Psidium* tubes, brick red in the plantain, and unchanged in the others. The colour had diffused into the water. The aerial growth was turning brown on the surface in the carrot, onion, plantain, *Colocasia*, *Carica*, *Psidium* and bread tubes, with a little pink in places in those of *Colocasia*. The medium was blackened in the potato, carrot and *Carica* tubes, while the upper part of the rice was dark coloured and a bluish line separated this from the unaltered lower portion ; the same bluish colour developed in the upper part of the bread paste. Seventeen days later there was little change, the growth in the potato and onion tubes was darker, that in the carrot, plantain, *Carica* and *Psidium* tubes had become pinkish in places, the submerged mycelium was chocolate-brown in the plantain, and chestnut in one of the sugarcane tubes, and a few dark dots were noticed in the denser parts of the mycelium of several tubes. These did not develop further and were ultimately found to be merely condensations of sterile mycelium.

The following inoculations were carried out with pure cultures, obtained as above described.

In October, 1909, 35 canes in a plot of strong healthy Red Mauritius, about 7 months old, were inoculated at Samalkota by removing a cylinder, about half an inch in length, with a small, flamed cork borer and inserting a small tuft of mycelium. The cylinder was replaced after cutting a little off the end, and the wounded stem bound with sterile gutta-percha sheeting. Seven weeks later, two of the inoculated canes commenced to wither. In January, 1910, another inoculated cane withered and was sent to Pusa for examination. The condition of the stem was similar to that in the canes from which the fungus was first obtained. The inoculated internode was bright red. The reddening extended upwards for 3 internodes, the 3rd having only one bright red bundle. Higher up, the translucent, watery condition of the pith, already described, was found. Downwards, the reddening extended for 2 internodes. Hyphæ were numerous in the reddened parts and pure cultures of the fungus used in inoculating were obtained readily. In February, four more of the withering inoculated canes were received, as well as two non-inoculated canes arising from the same clumps, which seemed to have become infected secondarily. The inoculated canes had typical symptoms. In two, the infection had extended downwards to the base of the stool, the upper part being less affected. In the other two, distinct reddening occurred for 2 or 3 internodes above the wound and the pith was translucent and hollow in the centre for some 10 internodes higher. The hyphæ were, as usual, confined to the reddened portion, where they were plentiful and easily obtained in pure culture, giving rise to the same fungus used in inoculating. The two non-inoculated canes had become infected from below ground, apparently through the roots, and were withering as a result of the attack. The fungus was recovered from these canes also. Early in March the plot was inspected by one of us (E. J. B.). A number of the inoculated canes had withered and the disease had extended in some cases to other canes in the same clumps. The rest of the plot was quite free from disease, except where the second series of inoculations, to be described below, was located. The symptoms were typical and left no doubt of the success of the inoculations.

On the same date as the last, a second series of inoculations was carried out in the same plot, by removing the soil from around the base of four clumps of cane and watering the exposed roots with a suspension of the mycelium beaten up in water. These also took well, and when seen early in March each clump had one or more withered canes. No other canes were withering in the rest of the row to which the clumps belonged nor, so far as could be observed, in any part of the plot, except the row in which the first series of inoculations had been made. One cane from each clump was split and found to have typical symptoms of the disease, but there was no opportunity for microscopic examination. The symptoms were, however, so definite that there was no doubt that this series was quite as successful as the first.

As the disease does not occur at Pusa, it was not considered advisable to carry out any inoculations in the field. Some were, however, made on plants growing in tubs, at a distance from the farm crop. The number of canes inoculated through stem wounds was six, growing in four tubs, one tub being kept as a control. The inoculations were made in November 1909, exactly in the same manner as those of the first series at Samalkota. In the following March none of the inoculated canes had withered and two were removed for examination. The inoculated internode was found diseased in both, the mycelium being confined to the one internode in one case but extending to the next higher up in the other. The other plants were kept in the tubs until the following year, by which time they were still alive but had ceased growth, except for a few feeble side shoots. In May 1911, they were cut and examined. The inoculated canes were much reddened near the wound, the reddening extending for from 2 to 4 internodes each way. Above the reddened part, the characteristic translucent appearance and internodal hollowing of the pith was found. The red parts were full of the hyphæ of the parasite and, in one case, pycnidial stromata had developed in the internode next below that inoculated. The roots were not affected and no spread to other shoots in the same stool had occurred. The parasitism was, therefore, much less marked than at Samalkota, though the spread of the

mycelium in the living tissues indicates a certain degree of parasitic activity.

In three other tubs, the main canes of which had been injured and in which a "ratoon" crop was growing, inoculations similar to those of the second series at Samalkota, were made in December 1909, the exposed roots being watered with a suspension of the fungus. No outward sign of disease appeared, and the following April the stools were uprooted, as also that in the control tub. All were found perfectly healthy and no trace of the parasite could be detected in the tissues.

It seems probable, therefore, that the disease is restricted in its distribution by climatic or other unknown conditions, as so many of the fungus blights of crops in India are. How much damage is caused by it is unknown. Both at Samalkota and Jorhat it was enough to cause uneasiness, but in the latter case it is still uncertain whether there are not two diseases at work. If it should be found that the condition observed at Jorhat, in the Central Provinces, and elsewhere, of excessive tillering, combined with degeneration of the plant, sometimes to such an extent that no true canes at all are formed, the whole plant remaining grass-like, is caused by this parasite, then it will have to be reckoned as a serious disease. Meanwhile, further investigations are in progress and it is hoped that the publication of the present description will lead to the recognition of the disease, where it occurs, and fuller observations on its field characters. It is obviously impossible, at present, to suggest methods for its check beyond the ordinary precautions urged on previous occasions for routine observance in sugarcane cultivation in India.

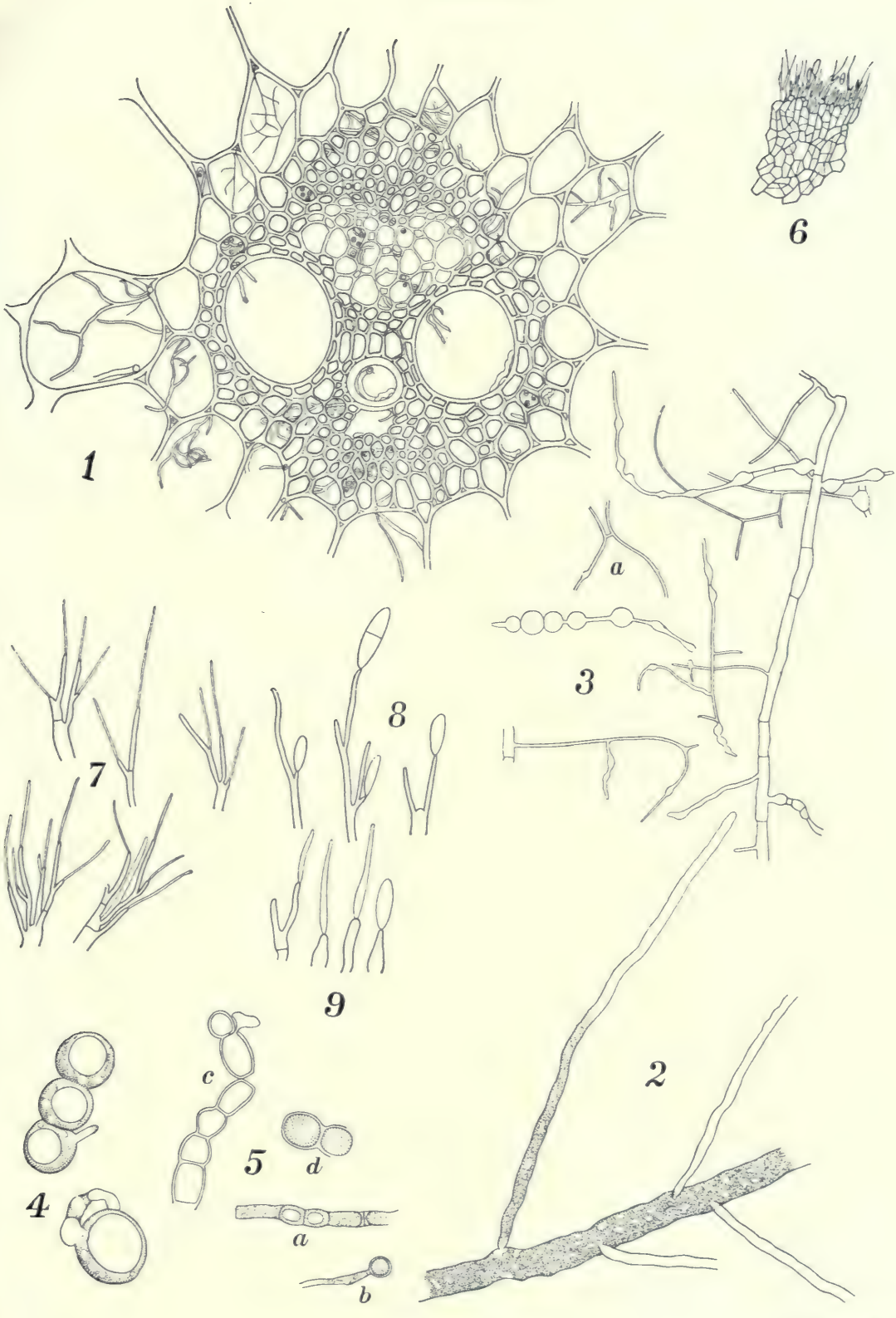
Description of Plates III, IV and V.

(*Hendersonina Sacchari*, Butl.)

Plate III.

Fig. 1. Section of a sclerotium of *Hendersonina Sacchari*, probably formed by the union of several unilocular stromata. Each cavity is surrounded by a separate pseudo-parenchymatous wall, between which the structure is filamentous. A mass of spores of both types is extruding from the mouth of the left-hand cavity.

PLATE IV.



HENDERSONINA SACCHARI.

Hendersonina Sacchari.

Photo. Engraved & printed at the Offices of the Survey of India, Calcutta, 1915

Fig. 2. Ripe sclerotium in longitudinal section, showing the irregular loculi, immersed in a pseudo-parenchymatous stroma. The cavities were filled with spores, which are not shown.

„ 3. Another ripe sclerotium in transverse section. At the base, this sclerotium showed only two large loculi. Near the apex there were seven cavities, arranged roughly in a ring and mostly opening by a common mouth.

Plate IV.

Fig. 1. Transverse section of part of the pith of a cane, infected by *Hendersonina Sacchari*. The hyphæ grow from the intercellular spaces into the cell cavities. Intermediate and fine hyphæ are present, but not the very thick kind. X 190.

„ 2. Very thick hypha from the surface growth obtained on incubating an inoculated cane. Branches of intermediate diameter are numerous. These in turn will give off very fine threads. Septation has not yet occurred. X 350.

„ 3. Mycelium from a culture, showing fine and intermediate hyphæ and chlamydospores. The fine branches are irregularly swollen, and at *a*, two have anastomosed by a side branch. Later stage of growth than Fig. 2. X 350.

„ 4. Chlamydospores separated from the mycelium and germinating. X 500.

„ 5. Formation of chlamydospores. *a* early stage, intercalar ; *b* ditto, terminal ; *c* a chain separating from the mycelium (one is germinating). *d* old chlamydospores from a chain, that on the right has lost part of its exospore. X 350.

„ 6. Part of the wall of a pycnidium, showing the abrupt transition from the sclerotial cell layers to the basidial layer.

„ 7. Basidia with filamentous spores (scolecospores). X 625.

„ 8. Basidia with the broad type of spore (pycnospore). X 625.

„ 9. Simple types of basidium, the 3 on the left with hyaline scolecospores of a broader type than usual, that on the right with a young pycnospore. X 625.

Plate V.

Fig. 1. Pycnospores of *Hendersonina Sacchari*. X 520.

„ 2. Scolecospores of ditto. Some stromata contain only the narrow type, uniform in diameter shown at *a a*, others the more irregular forms. X 520.

„ 3. Intermediate type of spore between the pycnospore and scolecospore types, X 520.

„ 4. Germination of the pycnospores, early stages (24 hours after sowing). X 520.

„ 5 Ditto, later stages (48 hours after sowing). X 520.

III.—Helminthosporiose

(*Helminthosporium Sacchari* Butl. n. sp.).

A species of this well-known genus, many members of which are parasitic on *Gramineæ*, is common on the leaves of sugarcane at Pusa. The infected leaves first show small red spots, which spread rapidly, chiefly in a longitudinal direction and, especially towards the tip of the leaf, may run together to form long streaks. The centre of the spot soon changes to a dirty straw colour, around which the margin remains red for a time and then changes to dark brown. The spots occur equally on the midrib, where they may be confused with those caused by the leaf form of *Colletotrichum falcatum*,* and on the thinner part of the leaf. When numerous, they cause death of the leaf tissues beyond the limits of the spots; the tip of the leaf often withers completely and there may be long withered strips down the margins.

The mycelium of the parasite is found in the leaf cells of the spotted portion and also collects in small stromatic masses on the surface of the spot. The hyphæ are brown at the surface and in the outer cell layers, but hyaline deeper in. They pass from cell to cell through narrow cracks in the walls (Pl. VI, Fig. 2), which are especially noticeable in the thick-walled sclerenchyma which overlies the bundles, but within the cells are swollen so as almost to fill the cavity in the smaller cells. In the epidermis they frequently form small stromatic masses. The cells appear to be killed in advance of the growth of the fungus, as although the hyphæ are numerous in the dead cells, it is rare to find penetration of a still living cell.

As soon as the centre of the spot begins to turn straw-coloured, fructification occurs by the growth of sporophores from

* Butler, E. J. & A. Hafiz Khan. Red Rot of Sugarcane. Mem. Dept. of Agric. in India, Bot Ser., VI, No. 5, 1913.

the stromata, both those within the epidermal cells and those on the surface of the leaf.

The sporophores are stout, erect, rather rigid hyphæ, which arise from the peripheral cells of the stromata (Pl. VI, Fig. 4). They are usually unbranched, 3 to 10 septate, dark greenish-brown below, paler above and several times bent or "geniculate." Spores are produced at each bend and at the apex, the lowest being the first formed and the bent condition being due to the spores being always apical at first and being then pushed to one side by continued growth of the sporophore from just below the insertion of the spore. The sporophores are 100 to 190 μ long, by 5·5 to 7·5 μ broad.

The spores (Pl. VI, Fig. 5) are borne singly and readily fall off. They are cylindrical or long elliptical in shape, with very thick walls, and divided into from 4 to 11 compartments by broad partitions. The colour varies from olive green to brown and the size from 35 to 60 μ long, by 8·5 to 12 μ broad.

Germination occurs rapidly (within 3 hours in some cases) by the protrusion of a germ-tube from each end of the spore. At the same time the internal partitions sometimes break down in the centre (Pl. VI, Fig. 6). Part of the mycelium formed from a single spore culture in a hanging drop, is shown in the text figure;

drawn 48 hours after sowing. The fungus can be readily cultivated on most ordinary media, but the spores formed in culture are usually smaller than those from the host plant. The following inoculations were made with pure cultures on nutrient agar.

In January 1909, 8 leaves of growing sugarcane were inoculated on the upper surface with a suspension of spores and mycelium, a drop of which was placed on each leaf and covered with damp, sterile cotton-wool. The leaf was not injured in any way. After 6 days, 3 of the inoculations were found to have succeeded, a red spot developing at the inoculated point and eventually giving rise to a typical infection. The other 5 and the controls showed no change. A second series was made at the same time on young healthy shoots brought into the laboratory. On these 6 inoculations were made, 3 being kept damp with cotton-wool and 3 by covering with bell-jars. The latter showed evident signs of infection in 2 days, but only 1 of the former succeeded and the spot was not visible until the fourth day. A month later 9 more inoculations were made on healthy shoots kept under bell-jars, all of which succeeded. One showed reddening in 24 hours, 4 more were visible in 3 days, and all were well marked in 5 days. A little later, 21 more inoculations were made in a similar manner to those of the last series. All succeeded well.

The microscopic examination of the inoculated leaves showed that penetration occurred directly into the epidermal cells (Pl. VI, Fig. 3). The infecting hypha swells up slightly in close contact with the cuticle and a narrow tube arises from the swollen portion and pierces the wall. As soon as the cell cavity is entered, the hypha swells up again and becomes freely septate, often forming a small mass of pseudo-parenchymatous cells. From this, branches pass to the deeper cells, and others approach the surface to form new stromata. The pressure exerted by these epidermal stromata is sometimes enough to rupture the outer wall before reproduction begins. The fructifications are usually confined to the quite dead central part of the spot.

The species does not seem to agree with any of those previously described. About 30 are known on various grasses, but none on *Saccharum*. The diagnosis is as follows :—

Helminthosporium Sacchari Butl. n. sp. Maculis amphigenis, elongatis, initio rubris, dein avellaneis vel stramineis ac ferrugineo-marginatis, 3-25 ⩒ 2-6 mm; cæspitulis minutis, atris; hyphis fertilibus erectis, simplicibus, 3-10—septatis, geniculatis, olivaceo-brunneis, apice pallidioribus, 100-190 ⩒ 5·5-7·5 μ; conidiis acrogenis, cylindraceis vel oblongo-ellipticis, utrinque rotundatis, 3-10-septatis, crassissime tunicatis, olivaceo-brunneis, 35-60 ⩒ 8·5-12 μ.

In folis *Sacchari officinarum* in India, or

A fungus which, from the published descriptions, bears a considerable resemblance to the above, is known in Java and Hawaii under the name of *Cercospora Sacchari*, Br. de Haan. It was first described by Breda de Haan in Java,* as the cause of a leaf disease to which the name "eye spot" was given. A further account was subsequently published by Dickhoff and Hein†. In Hawaii it has been briefly described by Cobb‡. From the figures published in Wakker and Went's well-known text-book of sugarcane diseases§, it appears probable that this fungus is really a *Helminthosporium* and not a *Cercospora*. A comparison of the two fungi has not been possible and could alone settle the question of their identity.

Little requires to be said regarding the treatment as, so far, this disease has not been found to damage the crop materially. If it became severe, it would be advisable to strip off and destroy the affected leaves in the early stages of the attack (we have found the first spots in June, at Pusa). There are a good many

* Breda de Haan, J. Van. Rood Rot en andere Ziekten in het Suikerriet. Meded. van het Proefstation West Java, XVI, 1892.

† Dickhoff, W. G. & S. A. Arendsen Hein. Eenige Waarnemingen omtrent de Oogvlekkenziekte. Archief Java Suikerindustrie, IX, 1901, p. 865.

‡ Cobb, N. A. Fungus Maladies of the Sugarcane. Exper. Stat. of the Hawaiian Sugar Planters' Association, Division of Pathology and Physiology, Bull. No. 6, 1909, p. 36.

§ Wakker, J. H & F. A F. C. Went. De Ziekten van het Suikerriet op Java. Leiden. 1898, Pl. XXI, figs. 1—5.

leaf diseases of sugarcane, but they cause little loss as a rule and, even in more advanced countries than India, do not seem to require treatment.

PUSA,
August 5th, 1913.

DESCRIPTION OF PLATE VI.

(*Helminthosporium Sacchari* Butl.)

Fig. 1. Section through leaf of sugarcane, showing hyphæ of *Helminthosporium Sacchari* in the outer cells. X 350.

,, 2. Part of a similar section, to show passage of the hyphæ from cell to cell. X 500.

,, 3. Infection of upper surface of leaf, from an inoculation, showing penetration of the outer wall of the epidermis and formation of small stromatic masses in the epidermal cells. X 930.

,, 4. Conidiophores with young conidia. X 350.

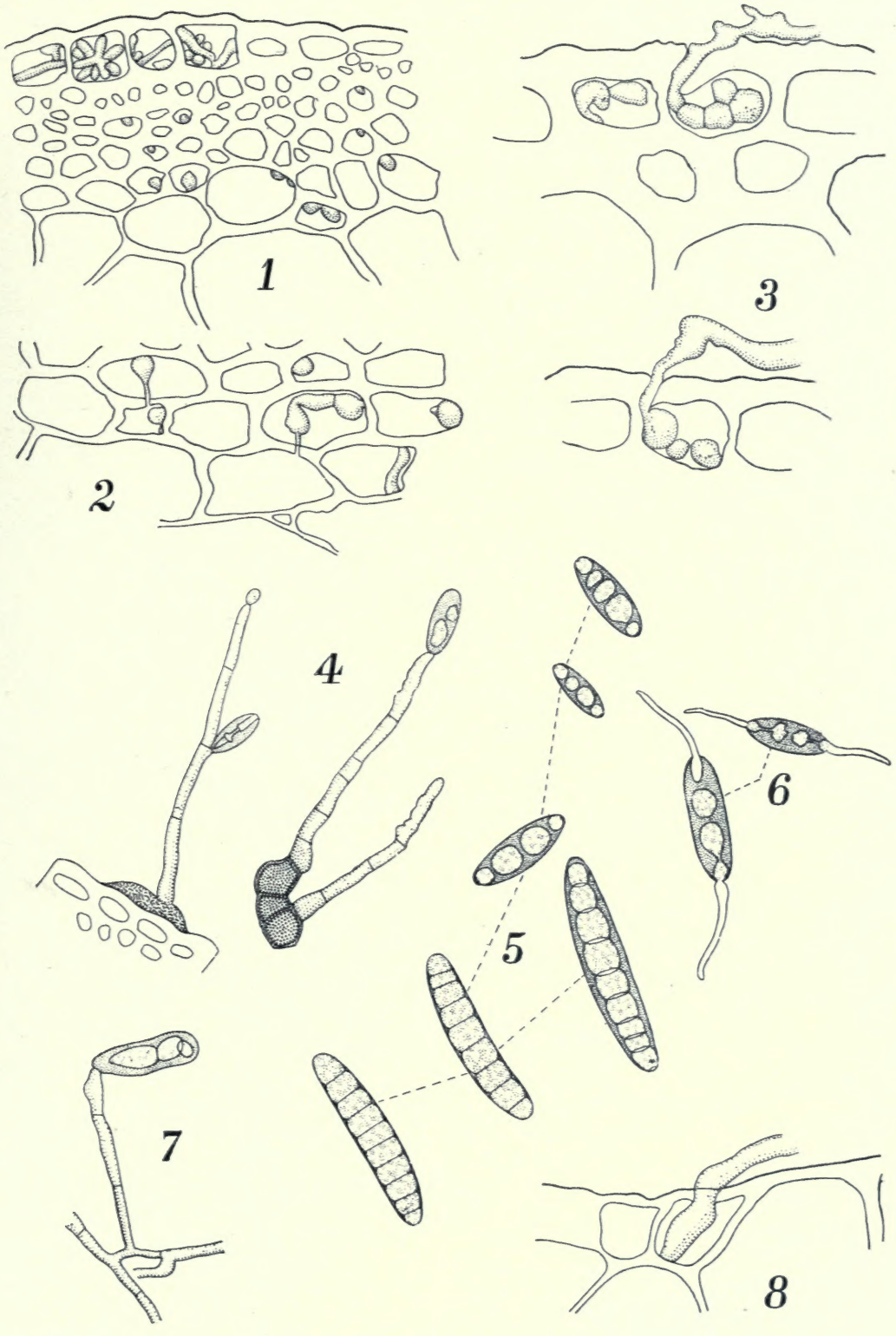
,, 5. Conidia. The three upper immature, the three lower mature. X 500

,, 6. Germination of the conidia. X 500.

,, 7. Reduced conidiophore and conidium, from a pure culture. X 350.

,, 8. Base of conidiophore, arising from within the epidermis and emerging across the cell wall, not through a stoma. X 500.

PLATE VI.



K.D.Das.Cop.

HELMINTHOSPORIUM SACCHARI.